Review

IMPORTANCE OF EPIDERMAL LIPIDS FOR PROPER FUNCTIONING OF THE STRATUM CORNEUM BARRIER

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SUMMARY

Proper structure of stratum corneum (SC) is important for regulation of vital biologic functions as are protection from penetration of foreign substances through the skin, protection from UV rays, abnormal transepidermal water loss (TEWL), protection from injuries, or smooth shedding of corneocytes. Two systems, the epidermal lipids (interstitial lipids of SC) and the corneocyte cell envelopes (CE) are primarily responsible for the so-called barrier function. Lipids reach the SC through the secretion of lamellar (Odland) bodies situated in the granular layer, while the CE are being formed from loricrin, involucrin as well as from other protein precursors. Barrier function is impaired in a number of skin disorders, e.g. chronic eczema, atopic dermatitis, ichthyoses, aged skin as well as in others. Clinical tests in humans and animal experiments are teaching us that the barrier function may be restored more quickly by applying ointments and creams containing adequate mixtures of lipids.

KEY WORDS

barrier function, stratum corneum, epidermal lipids, cornified cell envelope

INTRODUCTION

Dry skin and associated metabolic abnormalities are key symptoms in a number of skin disorders: cutis senilis, dermatitis atopica, contact dermatitis, ichthyoses, and others. A primary factor causing dry skin is the increased evaporation of water through the epidermis (transepidermal water loss, TEWL).

Proper functioning of stratum corneum (SC) is important for a number of processes, e.g. regulation of TEWL, inhibition of penetration of foreign substances through the skin, protection against UV irradiation, cooperation in the normal process of keratinization as well as adhesion and physiologic desquamation of korneocytes. This so-called *barrier function* depends on the integrity of the SC, specially on its content of lipids and on a properly structured cornified cell envelope (CE). TEWL can be measured and thus represents a model for assessing the barrier function of the skin.

The present is a rather simplified attempt to explain shortly and in a way understandable to clinicians the essentially very complicated premises for an adequate barrier function.

EPIDERMAL LIPIDS

Skin surface lipids derive in principles from two sources: firstly, lipids produced by sebaceous glands and secondly epidermal lipids which are an important constitutive material of the epidermal cells (cell membranes) and are mainly responsible for maintaining the barrier homeostasis.

Epidermal lipids are built up from a few basic substances which can be arbitrarily differentiated into backbone substances e.g. glycerol, sterols, sphingosine and ligands e.g. fatty acids, glucose, sulfuric and phosphoric acids. Ligands are esterified to the functional groups of the backbones in various combinations.

For didactic purposes epidermal lipids can be divided into three classes (1):

1. simple lipids like n-alkanes, fatty acids, triglycerols, phospholipids

2. sphingolipids are built up from the backbone sphingosine and the ligands fatty acid and glucose (ceramides and glucosylceramides)

3. sterols are synthesized by a different pathway than the other two classes. Free sterols, sterol esters, and cholesterol sulfate are characteristic compounds of epidermal lipids. Squalene, an intermediate in sterol synthesis, is a nonpolar sebaceous lipid on the skin surfaces, but it is only a minor component of epidermal lipids. Figure 1.

In parallel to the differentiation of keratins from basal layer to the stratum corneum, the composition of epidermal lipids also undergoes changes (1). In the basal layer polar lipids, e.g. phospholipids and glycosylceramides, are expressed in addition to neutral lipids, while nonpolar lipids as neutral lipids, ceramides and cholesterol are the main components of the outer SC. Actually, the glycosylceramides increase up to stratum granulosum (SG) and then rapidly decrease due to the activity of the glycosidases. Cholesterol sulfate also increases up to SG and then diminishes in response to the activity of steroid sulfatase. It may be concluded that some essential changes and events occur at the interface between the SG and SC providing the biochemical alterations as well the deposition of the lipids into the intercellular space (1,2,3). Figure 2.

In living epidermal cells the lipids are mainly localized in the cell membranes. In the SG they are present in the lamellar bodies (Odland bodies, LB)

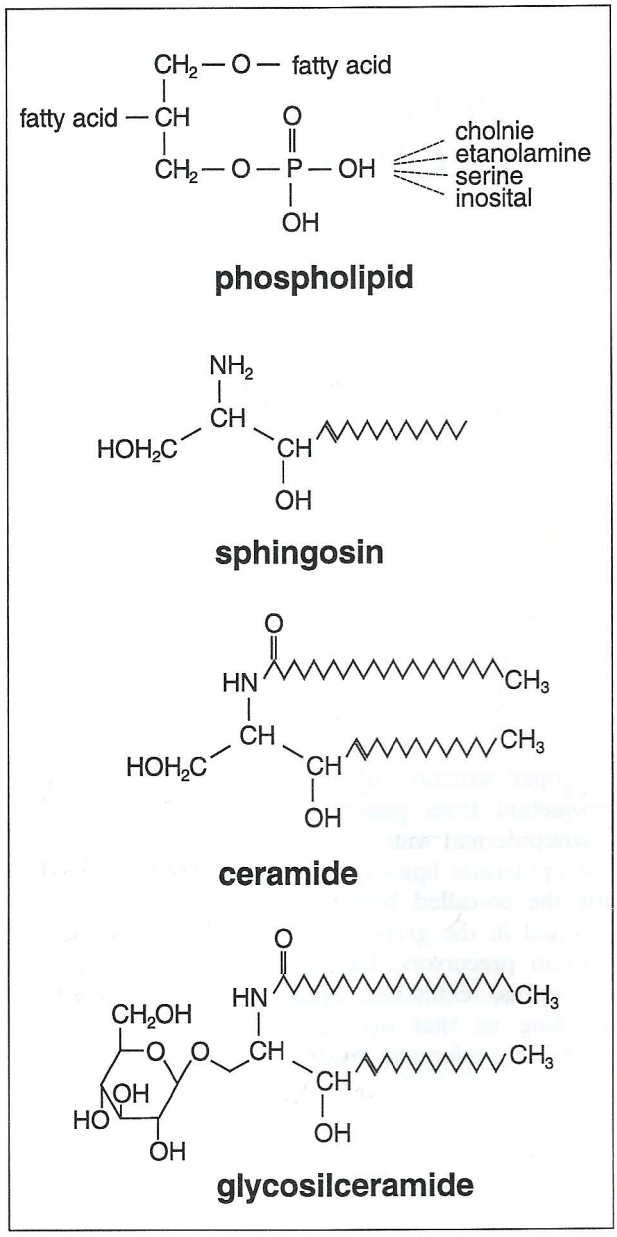

Figure 1. Chemical formulas of some epidermal lipids

and in the SC within the intercellular spaces as parallel lamellar structures (intercellular lipids). LB are specialized lipid storage or secretory organelles that are surrounded by a membrane and have a core composed of multilamellar membranes. The current concept represents SC as a two component system in which cells (corneocytes) are compared to bricks and intercellular lipids to mortar (4). The intercellular lipids are mainly expressed as lamellar bilayer structures by electron microscopy, using the ruthenium tetroxide postfixation. They are mainly responsible for controlling

the penetration of exogenous substances through the skin as well as for controlling the TEWL. The intercellular lipids of SC derive mostly through the secretion of the LB. Figure 3.

The best-investigated LBs are produced by pneumocyte II cells in the lung alveoli where they provide the surfactant system (5).

Under experimental conditions the barrier function may be impaired or eliminated by tape stripping or extraction with solvents. In the young, the epidermis barrier perturbation results in a homeostatic response, which includes: 1. immediate secretion of already existing lamellar bodies, 2. new formation and ongoing secretion of lamellar bodies. This secretory response is fueled by increased synthesis of cholesterol, fatty acids, and ceramides. Increased levels of 3-hydroxy-3 methylglutaryl-coenzyme A reductase (HMG-CoA red) which is the rate-limiting enzyme of cholesterologenesis, as well as an increase in the activities of acetyl CoA carboxylase and fatty acids synthase which are the rate-limiting enzymes of fatty acids synthesis, are responsible for the restitution of the intercellular lipids (6).

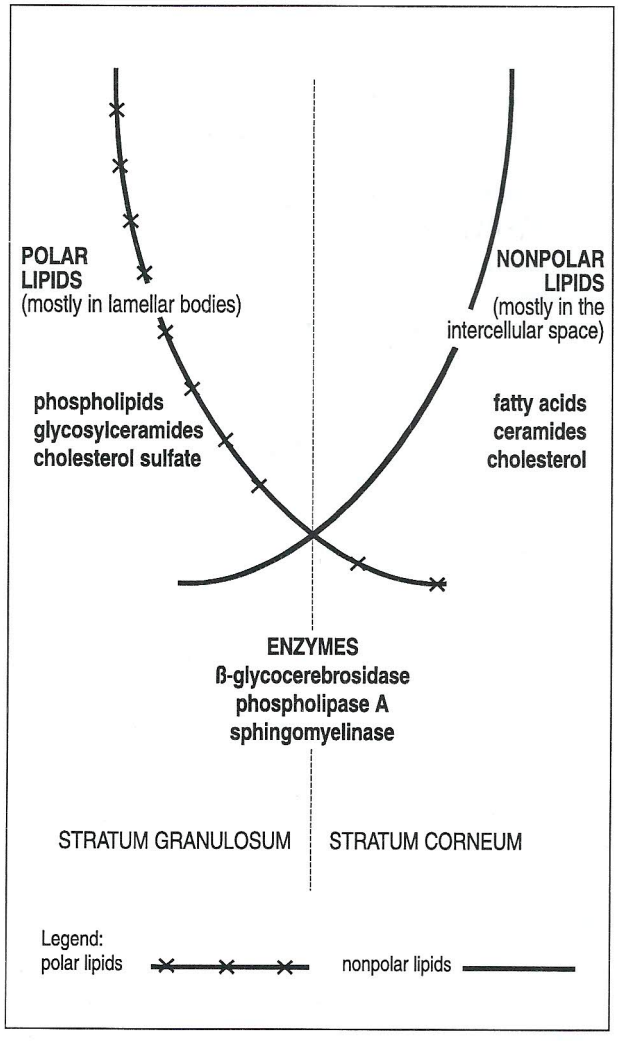

Figure 2. Distribution of lipid fractions in stratum granulosum and stratum corneum

In aged persons the SC displays a global reduction in lipids with reduced number of extracellular bilayers. The permeability of the barrier is adequate under basal (normal) conditions, but when challenged acutely, its integrity and recovery are impaired in both aged human and aged murine skin (7). In a similar way the barrier recovery was delayed in aged mice after a chronic perturbation due to a reduced synthesis of cholesterol in response to a decreased activity of HMG Co A red (8).

CORNIFIED CELL ENVELOPE

The final stage of mammalian epidermal cell differentiation is the formation of the cornified cell envelope (CE), which is a 15 nm thick cross-linked sheath of proteins, on the inner surface of the plasma membrane in keratinocytes of the stratum corneum (9,10). The CE is built from cross-linked glutamate and lysine isopeptides. The epidermal enzyme transglutaminase (TGM) plays an important role by catalyzing the calcium dependent cross-linking of proteins. It seems that the assembly of CE from its precursors is a gradual and complex process, in which numerous components participate, contributing to its thickness and rigidity. The best-characterized components (precursors) are loricrin (LOR), involucrin (INV) and the small prolin-rich proteins (SPRPs), all of them encoded by genes mapping to a region of chromosome 1q 21, known as epidermal differentiation complex (EDC) (10).

Other known CE precursor proteins, which map to the same EDC region, are profilaggrin (proteolytically processed into filaggrin and serves as a matrix for packing intermediate filaments inside the corneocytes), trichohyaline and the small calcium binding proteins (S100A1-S100A11).

Desmosomal components as desmoplakin I, plakoglobin, envoplakin, plakophilin, desmocollin 3a/3b, desmoglein 3 are also active in structuring the CE. Further substances structuring the corneocyte and the CE are keratins (intermediate filaments), plasmogen activator inhibitor (PAI-2), annexin, as well as other less defined proteins (10,11). Figure 4.

The newly synthesized proteins undergo post synthetic fatty acid acylation resulting in attachment of fatty acids and ceramides derived from LB to the surface of the envelope (12).

A certain number of mutations involving the CE have been identified. Defects of TGMs causing lamellar ichthyosis (LI) are most frequently mentioned in the literature (13,14). A hereditary molecular defect of loricrin has also been described as the cause of the Vohwinkel syndrome (11).

BARRIER FUNCTION

If the stratum corneum is exposed to solvents, they dissolve the intercellular lipid structures and externally applied substances easily penetrate through it. At the body sites with a lower lipid content a better permeation of substances from outside and an increased TEWL may be observed.

The regulation of TEWL was investigated years ago by Elias and his group (15) by introducing the essential fatty acids deficiency (EFAD) model in mice. Mice on an EFAD diet develop a hyperkeratosis of proliferative type and the LB appear largely empty as observed by electron microscopy. With a longer duration of this diet an increased TEWL was observed, which could be abolished by topical or systemic application of linoleic acid, but this did not abolish the hyperproliferative state. Hyperproliferation was ameliorated by arachidonic acid, which did not influence the TEWL. It may be deducted from these animal experiments, 1. that in pathological conditions in which barrier function is defective, such a condition can be provoked by an inadequate supply of essential fatty acids (linoleic acid), and 2. that a lack in arachidonic acid can be the cause of epidermal hyperproliferation. Cholesterol also plays an important role in the proper functioning and maintenance of the epidermis and especially the horny layer. Diazocholesterol, which substance inhibits the cholesterol synthesis, induces in hairless mice a retention type hyperkeratosis, similar to that observed in x-linked recessive ichthyosis (16). The water-binding capacity of stratum corneum depends mainly on the presence of ceramides and further lipids, though other natural moisturizing factors, e.g. amino acids, sugars as well as other molecules are also important in this respect (17). It is not probable that the thin layer of emulsified fat on the skin surface, which results from the secretion of sebaceous glands (lipid film), has any great effect on the skin permeability.

An increased TEWL compared to normal volunteers was reported in 3 patients with autosomal recessive erythrodermic lamellar ichthyosis (18). Such an observation was linked to a significant difference in

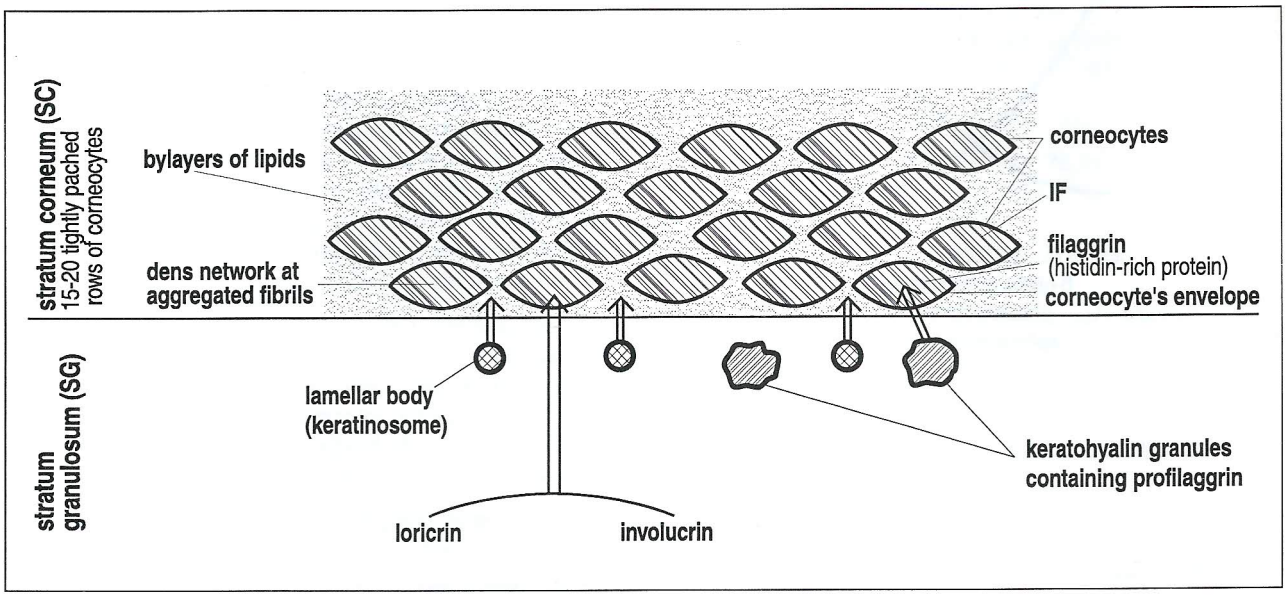

Figure 3. Schematic presentation of human stratum corneum (brick and mortar model). Intercellular lipid bylayers are formed mostly through secretion of lamellar bodies.
Legend: CE - cornified cell envelope, SC - stratum corneum, SG - stratum granulosum, IF - intermediate filaments

the relative amounts of ceramides and other lipids. Abnormalities in epidermal lipid metabolism were also reported in-patients with atopic dermatitis (19).

EPIDERMAL LIPIDS IN DISORDERS OF KERATINIZATION

Many of the pathogenic mechanisms triggering ichthyotic manifestations are still not known. Following data result from molecular biology investigations and from analysis of lipids in the ichthyotic scales. Metabolic defects were best investigated in x-recessive

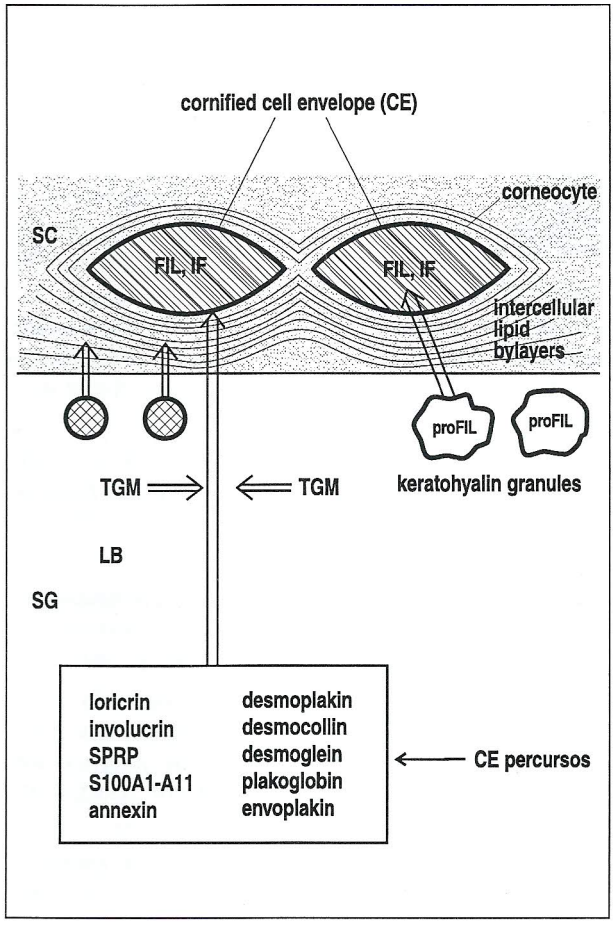

Figure 4. Schematic presentation of the cornified cell envelope formation
Legend: SC - stratum corneum, proFIL - profilaggrin, SG - stratum granulosum, SPRP - small prolin rich proteins, CE - cornified cell envelope, S100A1-A11 small calcium binding proteins, FIL – filaggrin, LB - lamellar bodies, IF - Intermediate filaments, TGM - transglutaminase

ichthyosis vulgaris. In 1978, Shapiro et al have demonstrated the deficiency of the enzyme steroid sulfatase in cultured fibroblasts from such patients (20). Later on, such a defect was detected also in leukocytes and keratinocytes. As a consequence of the mentioned enzymatic deficiency cholesterol sulfate, which is normally catabolized to cholesterol, is increased in the outer SC, while free cholesterol is decreased (21). This metabolic defect seems to be corresponsive for the adherence of ichthyotic scales. An increase in LDL cholesterol fraction in plasma has also been reported in these patients (22).

Erythrodermia ichthyosiformis congenita (erythrodermic lamellar ichthyosis) is characterized by an increased content of n-alkanes in the horny layer (23), however such findings are not specific as they may be found also in neutral lipid storage diseases. In Refsum's syndrome the accumulation of phytanic acid due to a deficiency of phytanic acid alfa hydroxylase is responsible for the disturbed keratinization. A linkage between nonerythrodermic lamellar ichthyosis and an increase of cholesterol in the epidermis is also probable (1).

In harlequin ichthyosis there is an abnormal expression of keratins and fillagrin, in addition lamellar bodies and intercellular lipids within the SC are absent or abnormal (24). There is evidence that the harlequin ichthyosis belongs to a genetically heterogeneous group of disorders.

In certain patients with lamellar ichthyosis (LI) deficiency in transglutaminase 1 (TGM-1) was detected. Permentier et al observed out of 23 families with severe LI a linkage to TGM-1 in 10 families, while in 13 such linkage did not exist (13).

It was already mentioned that in the Vohwinkel syndrome (keratosis palmoplantaris mutilans) a molecular defect of loricrin has been reported (11).

CONCLUSION

A short review of certain pathological mechanisms contributing to the dry and to ichthyotic skin was attempted. Available experimental data suggest that an impaired barrier function is responsible for dry skin. Impaired metabolism of lipids in the skin and especially in the SC plays a major role in causing ichthyosis. Animal experiments as well as studies in human skin have provided many interesting data, it seems however that many more efforts are needed to solve all the puzzles involved in the pathogenesis of ichthyosis. Some of the newly discovered facts

concerning the barrier function of SC represent a valuable contribution in treatment of certain skin diseases (25). Linoleic acid, ceramids, cholesterol, and some other ingredients are being currently incorporated in commercially available topical preparations.

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